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EXAMINER	
SINGH, ANOOP KUMAR	
ART UNIT	**PAPER NUMBER**
1632	

DATE MAILED: 11/20/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/510,652	LIBUTTI ET AL.	
	Examiner	Art Unit	
	Anoop Singh	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 October 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *26-50* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *26-50* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *10/8/04;10/28/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election with traverse of the invention of claims 41-50 (group II) filed October 4, 2006 is acknowledged. The traversal is on the grounds that Examiner has not set forth convincing argument that the search and examination of all the groups necessarily represents an undue burden for the examiner. Applicants argument for examining remaining claims drawn to a method of measuring the angiogenic activity of a test molecule by comparing the fluorescence vascular density are persuasive since both sets of group embrace methods of measuring angiogenic or anti angiogenic activity. Therefore, invention of claims 26-40 (group I) directed to a method of measuring angiogenic activity by comparing the fluorescence vascular density assay is rejoined with elected inventions of group II for the examination purposes. Applicants have also elected polypeptide, synthetic molecule, fluorescein, XTT, serum and filter paper as species for claims readable on claims 26-50.

Claims 26-50 are under consideration.

Priority

It is noted that instant application is a 371 of PCT/US03/10932 filed on 04/09/2003 which claims benefit of 60/371,010 filed on 04/09/2002. However, upon review the disclosure of the prior-filed application, US provisional application

60/371,010, fails to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. It is noted that 60/371,010 dated 4/9/2002 describes measuring angiogenic activity using fluorescence vascular density but does not show support for a method to determine angiogenic activity using XTT. Consequently, there is no written description in application for using XTT or any other metabolic agent to determine angiogenic activity using spectrophotometer. In case, if applicants have evidence to support otherwise, applicants are invited to indicate page and line number for the written support as recited in claims 41-50 of the instant application. Therefore, the effective filing date for instant claims 41-50 is 04/09/2003 as subject matter of instant claims was described in the 60/371,010.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 26-50 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

(i) a method of measuring the angiogenic or anti angiogenic activity of a test molecule by measuring fluorescent vascular density in a chorioallantoic membrane (CAM) assay; said method comprising: (a) obtaining an 10 days old chicken egg, (b) creating a window in the shell of the egg, such that the CAM is exposed, (c) providing to a test region of interest on the CAM a filter disk; (d) administering to a vessel located in

the CAM a test molecule, (e) administering to a vessel located in the CAM a fluorescent-labeled -dextran, such that the fluorescent-labeled dextran travels through each vessel contained in the test region of interest, (f) removing the substrate and the test region of interest from the fowl egg, (g) capturing a three-dimensional image of the test region of interest, wherein the three-dimensional image comprises a plurality of pixels, such that a fluorescent vascular density (FVD) value can be assigned to the test region of interest, and (h) comparing the FVD value of the test region of interest with the FVD value of a control region of interest that was prepared in the same manner as the test region of interest but without the administration of a test molecule or with the administration of a control molecule, such that the angiogenic or anti angiogenic activity of the test molecule is measured, wherein a lower FVD value of the test region of interest as compared to the FVD value of the control region of interest is indicative of the test molecule being useful as an inhibitor of angiogenesis, and wherein a higher FVD value of the test region of interest as compared to the FVD value of the control region of interest is indicative of the test molecule being useful as a stimulator of angiogenesis;

(ii) a method of measuring the angiogenic or anti angiogenic activity of a test molecule by measuring metabolic activity of cells in a chorioallantoic membrane (CAM) assay comprising: (a) obtaining an 10days old chicken egg, (b) creating a window in the shell of the egg, such that the CAM is exposed, (c) providing to a test region of interest on the CAM a substrate, (d) administering to a vessel located in the CAM a test molecule, (e) administering to a vessel located in the CAM an agent XTT to measure

metabolic activity, (f) removing the substrate and the test region of interest from the egg, (g) measuring the spectrophotometric absorbance value of the test region of interest at 450-500 nm wavelength, and (h) comparing the spectrophotometric absorbance value of the test region of interest with the spectrophotometric absorbance value of a control region of interest that was prepared in the same manner as the test region of interest but without the administration of a test molecule or with the administration of a control molecule, such that the angiogenic or anti angiogenic activity of the test molecule in CAM assay is measured, wherein a significant lower spectrophotometric absorbance value of the test region of interest as compared to the spectrophotometric absorbance value of the control region of interest is indicative of the test molecule being useful as an inhibitor of angiogenesis, and wherein a significant higher spectrophotometric absorbance value of the test region of interest as compared to the spectrophotometric absorbance value of the control region of interest is indicative of the test molecule being useful as a stimulator of angiogenesis

does not reasonably provide enablement for measuring angiogenic or anti angiogenic activity of a test molecule using any other substrate or fluorescent labeled particle or assigning any brightness to each pixel in the image for calculating FVD or using any agent to measure metabolic activity at any other wavelength. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

In determining whether Applicant's claims are enabled, it must be found that one of skill in the art at the time of invention by applicant would not have had to perform "undue experimentation" to make and/or use the invention claimed. Such a determination is not a simple factual consideration, but is a conclusion reached by weighing at least eight factors as set forth in In re Wands, 858 F.2d at 737, 8 USPQ 1400, 2d at 1404. Such factors are: (1) The breadth of the claims; (2) The nature of the invention; (3) The state of the art; (4) The level of one of ordinary skill in the art; (5) The level of predictability in the art; (6) The amount of direction and guidance provided by Applicant; (7) The existence of working examples; and (8) The quantity of experimentation needed to make and/or use the invention.

The office has analyzed the specification in direct accordance to the factors outlines in *In re Wands*. MPEP 2164.04 states: "[W]hile the analysis and conclusion of a lack of enablement are based on factors discussed in MPEP 2164.01(a) and the evidence as whole, it is not necessary to discuss each factor in written enablement rejection." These factors will be analyzed, in turn, to demonstrate that one of ordinary skill in the art would have had to perform "undue experimentation" to make and/or use the invention and therefore, applicant's claims are not enabled.

It is noted that independent claim 26 is broad in scope. The following paragraph will outline the full scope of the claims. This claim is broad in scope, encompassing a method of measuring angiogenic or anti angiogenic activity of any test molecule by obtaining any embryonatd fowl egg of any stage using any fluorescent-labeled particle excited by laser to any wavelength by assigning any brightness to each pixel for

calculating fluorescent vascular density (FVD). It is also noted that claim 41 embrace a method of measuring angiogenic or anti angiogenic activity of any test molecule by obtaining any embryonatd fowl egg using any agent to measure metabolic activity and measuring at any measuring absorbance to determine angiogenic or anti angiogenic activity. The disclosure provided by the applicant, in view of prior art, must encompass a wide area of knowledge to a reasonably comprehensive extent. In other word each of those, aspect considered broad must be shown to a reasonable extent so that one of the ordinary skills in the art at the time of invention by applicant would be able to practice the invention without any undue burden being on such Artisan.

The claims are directed to a method of measuring the angiogenic or anti angiogenic activity in a CAM assay. Claim 27 further limits the embryonated fowl egg to include chicken egg. Claim 28 limits the substrate to include glass, plastic and plurality of substrate including filter paper. Claims 29-32 include substrate comprising either stimulator or inhibitor of angiogenesis. Claims 33-34 limit the method to include steps wherein fluorescent-labeled particle and test substance is administered to different vessel in the CAM subsequently limiting to each vessel that is cannulated prior to administration of the test molecule and the fluorescent-labeled particle. Claim 35 limits the fluorescent-labeled particle to include fluorescent-labeled protein, polypeptide or polymer subsequently limiting to fluorescein, green fluorescent protein, yellow fluorescent protein, Lucifer yellow, rhodamine, cyanine based compounds, C6-NBD, DIO-Cn-(3), BODIPY-FL, eosin, propidium iodide, Dil-Cn-(3), Cy3, Texas Red, Dil-Cn-(5), allophycocyanin, and Cy5. Claims 37-39 limit the method to include fluorescent-

labeled particle that is labeled with a fluorescent moiety that can be excited by a laser and the three-dimensional image is captured by laser confocal microscopy, wherein the three-dimensional images are formed that are analyzed by computer analysis of the relative fluorescent brightness of each pixel to determine an FVD value for each vessel contained in each cross-sectional image. Claim 40 limits the method of claim 19 to include step to calculate FVD value of the region of interest. Claims 41-50 are directed to a method of measuring the angiogenic or anti angiogenic activity in a CAM assay. Claims limit the method to include agents such as XTT, MTT or WST-1, further limits the embryonated fowl egg to include chicken egg and substrate to include glass, plastic and plurality of substrate including filter paper. The method also include steps wherein agent and test substance is administered to different vessel in the CAM subsequently limiting to each vessel that is cannulated prior to administration of the test molecule and the agent to measure metabolic activity.

Claims 26-50 are directed to a method of measuring the angiogenic or anti angiogenic activity of a test molecule in a CAM assay. The specification and prior art teaches angiogenesis as a process by which new blood vessels form pre-existing vessels are formed (see specification, paragraph 2). It is also noted that specification describes angiogenesis as highly complex biological process that involves a number of different molecules and signaling pathways, the development of various strategies to study this process (see paragraph 3 of the published application). In addition, specification has exemplified a direct quantitative measurement of endothelial cell proliferation in the CAM assay by systemic injection of fluorescein isothiocyanate

(FITC)-Dextran (1 mg/ml), which was allowed to circulate for 30 minutes and then analyzing the image using a laser confocal microscope (see example 1 of the specification). In addition, specification has exemplified a direct quantitative measurement of endothelial cell proliferation in the CAM assay using an agent XTT to quantify endothelial cell proliferation or inhibition in the CAM assay (see example 2 of the specification). Prior art teaches that angiogenesis is the growth of new vascular capillary channels from pre-existing vessels, and is of fundamental importance in a number of physiological processes such as embryonic development, reproduction, wound healing and bone repair. Uncontrolled angiogenesis is pathological and is often associated with tumor growth, rheumatoid arthritis, diabetic retinopathy and hemangiomas (Folkman et al J Biol. Chem. 1992; 267(16): 10931-4). It is also noted that a number of methods are currently being used to measure angiogenic and anti angiogenic activity. Munoz-Chapuli et al (Cell Mol Life Sci. 2004; 61(17): 2224-43) describe "angiogenic signals promote endothelial cell proliferation, increased resistance to apoptosis, changes in proteolytic balance, cytoskeletal reorganization, migration and, finally, differentiation and formation of a new vascular lumen (see abstract)". It is emphasized that as recited these claims broadly read on measuring angiogenic or anti angiogenic activity of a test molecule. The specification discloses using fluorescein isothiocyanate (FITC)-Dextran (1 mg/ml) and measuring fluorescence by confocal microscopy or using an agent such as XTT by measuring the metabolic activity in the CAM assay for determining angiogenic or anti angiogenic activity. However, prior art teaches limitation of using CAM assay for measuring angiogenic or angiogenic activity.

For instance, Ribatti et al (The Anatomical Record 2001, 264, 317-324) state, "main limitation of CAM assay is the nonspecific inflammatory response including a secondary vaso proliferative response and impending quantification of the primary response. Ribatti et al further teach that other drawback of CAM assay is that test molecule is placed in the existing vessel and newly formed vessel grow within the CAM mesenchyme, thus the real neo vascularization can hardly be distinguishable from a falsely increased vascular density due to the rearrangement of existing vessel that follows the contraction of the membrane (see page 321, col. 2, para. 1). Ribatti et al emphasize the timing of the CAM angiogenic response is essential and noted that early angiogenesis after 24 hours only measures vasodilatation and not the angiogenesis. Therefore, any measurement of vessel density and vasodilatation and neo vascularization are not readily distinguishable and could only be overcome by sequential photograph (see 321, col. 2, para. 1 bridging to page 322). Thus, it is apparent that CAM itself is undergoing rapid changes both morphologically and in terms of the gradual change in the rate of endothelial cell proliferation during the course of embryonic development. Therefore, any measurement of fluorescence or metabolic product formazan of vessel comprising cells by using a fluorescent particle or XTT may provide result that may be because of vasodilation and not because of angiogenesis or because of changes in embryonic development. In addition, claims as recited do not recite specific stage of egg being used for conducting CAM assay which would further mask the effect thereby resulting in fluorescent or absorbance reading that would not correctly reflect the angiogenic or anti angiogenic activity as contemplated by the

specification. In absence of any explicit teaching, it is clear that instant method may not provide the real angiogenic activity rather it may only FVD or metabolic activity of cells in a CAM assay.

In conclusion, in view of breadth of the claims and absence of a strong showing by applicant, in the way of specific guidance and direction, and/or working examples demonstrating the same, such invention as claimed by applicant is not enabled for the claimed inventions. The specification and prior art do not teach method of measuring angiogenic or anti angiogenic activity as contemplated in the instant claims. An artisan of skill would have to perform undue experimentation to practice the method as claimed because the art measuring angiogenic activity in CAM assay using any fluoresce labeled particle or any agent having metabolic activity was unpredictable at the time of filing of this application as supported by the observations in the art record.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 26-27, 29-41 and 43-50 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to

reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claimed invention encompasses a method of for measuring the angiogenic or anti angiogenic activity of any test molecule in a CAM assay using any substrate and any fluorescent-labeled particle or any metabolic agent to determine angiogenic or anti angiogenic activity.

In analyzing whether the written description requirement is met for the genus claim, it is determined whether a representative number of species have been sufficiently described by other relevant identifying characteristics, specific features and functional attributes that would distinguish different members of the claimed genus.

This genus comprises any substrate or any fluorescent-labeled particle to capture 3D image of vessel to determine angiogenic or anti angiogenic activity. The specification teaches that the substrate for use in the instant invention can be any suitable substrate including glass, plastic, nylon, silicon, polytetrafluroethylene, a solubulized basement membrane preparation, collagen, fibrinogen, agarose, methylcellulose or filter paper (see paragraph 16 of the specification). In addition, specification discloses that any suitable fluorescent-labeled particle can be used in the instant invention including any suitable fluorescent moiety -labeled carbohydrate, a fluorescent-labeled protein, polypeptide, or peptide, or a fluorescent-labeled synthetic polymer (see paragraph 36). The specification also contemplates any suitable agent to measure metabolic activity can be used in the instant methods including XTT, MTT and WST-1 (see paragraph 11).

The specification does not describe the complete structure or any other physical or chemical specific structure or domain that are essential for a particle that would be suitable for labeling any fluorescent moiety or any other metabolic agent to measure angiogenic activity. A skilled artisan could not predict the structure of the any other agent nor could a skilled artisan predict the structure of all the substrate that could further comprise a test molecule. Since given the breadth of the claim, it could also encompass a substrate for an enzyme showing anti angiogenic activity. The specification does not provide any disclosure as to what would have been the required structure for active substrate or particle to be labeled with fluorescence moiety and whether such a substrate and particle would have desirable permeability properties in the vessel. Therefore, possession of filter paper and FITC-dexran does not predict the structure and characteristics of using any other substrate or any other fluorescent-labeled particle showing contemplated activity for capturing image to determine angiogenic activity. The specification also does not provide any disclosure as to what would have been the required structure for an agent that would have desirable permeability and solubility of resulting formazan product in the vessel. Therefore, possession of MTT, XTT or WST-1 does not predict the structure and characteristics of using any other agent showing metabolic activity that could be used in contemplated activity for measuring angiogenic activity.

Next, it is determined whether a representative number of species have been sufficiently described by other relevant identifying characteristics, specific feature and functional attributes that would distinguish different members of claimed genus. In the

instant case, the only identifying characteristic is that any suitable particle can be associated with any suitable fluorescent moiety depending upon device or any suitable substrate or any suitable agent can be used for determining metabolic activity could be used in instant invention. No other identifying characteristics of other substrates or particles or agents are disclosed. Further without a clear teaching of the essential elements of claimed substrates or particles and lack of identifying characteristics, a skilled artisan cannot envision the detailed structure of all the any other particles or substrates further comprising therapeutic agents or biological fluids that must show the contemplated biological activity. Therefore, conception is not achieved until reduction to practice has occurred, regardless of the complexity/simplicity of the structure and/or methods disclosed in specification.

In conclusion, this limited information is not deemed sufficient to reasonably convey to one skilled in the art that Applicant was in possession of all the other substrate and particle that could be used in the instant method showing contemplated biological activity at the time the application was filed. Thus, it is concluded that the written description requirement is not satisfied for the claimed genus.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 37-40 and 41-50 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The term " relative fluorescent brightness " in claim 39 is a relative term that renders the claim indefinite. The terms are not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. In the instant case, it unclear relative to what fluorescent brightness of each pixel would facilitate calculation of FVD value. The meets and bounds of the claim in not clear. Claims 40 depend on claim 39. Appropriate correction is required.

Claims 37-40 are vague and indefinite to the extent that these claims do not further limit the method of claim 1 which requires comparing FVD value of test agent with control FVD value. Therefore, method steps recited in claims 37-40 inherently must occur in order to determine FVD value. Appropriate correction is required.

The term "lower spectrophotometer absorbance and higher spectrophotometer absorbance" in claim 41 is a relative term that renders the claim indefinite. The terms are not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. In the instant case, it unclear how much lower or higher absorbance would be considered to have inhibitory or stimulatory effects? The meets and bounds of the claim in not clear. Claims 42-50 are directly or indirectly dependent on claim 41. Appropriate correction is required.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 26-34, 36-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Brooks et al; (Science, 1994, 264, 570-571 IDS) and Robert et al (Cancer Res. 1992; 52(4): 924-30) and Kimel et al (SPIE, 1996, 2628, 69-76, IDS).

Brooks et al teach a method to measure angiogenic and anti angiogenic activity of a test molecule by obtaining a 10 day old chick egg wherein it is candled to determine prominent blood vessel and then via a small window of exposed area a filter disc is placed followed by systemic administration of test molecule (see pages 570, Figure 2 A and B). It is noted that Brooks et al also suggest filter disc saturated with teat agent which could be angiogenic stimulator that could be placed on the CAM (see page 570,

Figure 2A, 3). Brooks et al also teach quantitation of angiogenic or anti angiogenic activity by removing the filter disc and associated CAM tissue that is snap frozen and sections that are stained with different antibody for staining of vessel which is analyzed using confocal microscopy (see page 570, Figure 2 A and B). Brooks et al analyzed average rhodamine fluorescence for each vessel per unit area to measure laser confocal image to determine angiogenic or anti angiogenic activity. While Brooks et al described the potential of measuring angiogenic and anti angiogenic activity using CAM assay and also disclosed quantified relative expression of integrins during bFGF induced angiogenesis by laser confocal image analysis (see Figure 3, page 571). Brooks et al differed from the claimed invention by not teaching administering fluorescent-labeled particle before removing the test region of interest and capturing the 3D image of the test region to quantitate angiogenesis in the test area.

However, prior to instant invention was made, Robert et al teach a method using chick embryo wherein different photo sensitizer including photofrin, cyanine and uroporphyrin were administered by different method including direct injection of photo sensitizer into the vessel to compare photo sensitizer uptake/retention in proliferating and non proliferating neovasculature (see abstract and page 925, col. 1, para. 4). The fluorescence is calculated using spectrofluorimeter to analyze photo sensitizer content (see page 925, col. 1, extraction). Robert et al provide evidence that photo sensitizers are preferentially retained by tumors have a selective affinity for proliferating neovasculature. It is noted that Robert et al disclose that the chloroaluminum sulfonated phthalocyanine and tetraphenyl porphine sulfonate compounds possess the greatest

affinity for proliferating neovasculature relative to nonvascular tissue (see abstract and Figure 2 and 3). This is further supported by studies conducted by Kimel et al that taught an *in vivo* uptake of porphyrin using CAM model to document fluorescence in real time at different time interval to demonstrate bio distribution of porphyrin (see abstract and conclusion).

Robert differed from the claimed invention by not teaching administering an anti angiogenic agent and measuring the fluorescence by confocal to measure the angiogenic or anti angiogenic activity. However, the use of confocal microscope for measuring the angiogenic activity was known in the art at the time the claimed invention was made and these were routinely used as evidenced by Brooks et al (supra).

Accordingly, in view of the teachings of Brooks and Robert, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the method to measure angiogenic or anti angiogenic activity in a CAM assay of Brooks by measuring fluorescent vascular density taught by Robert/ Kimel with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as Robert had already disclosed that phthalocyanine and tetraphenyl porphine sulfonate compounds possess the greatest affinity for proliferating neo vasculature relative to non vascular tissue (supra) and particularly since both Brooks and Kimel et al sought to quantitate retention of fluorescent moiety in CAM. Although Brooks et al did not adminster the fluorescent moiety before removing the substrate; he generally embraced potential of measuring fluorescence for quantification to better measure and compare angiogenesis. In

addition, Robert et al and Kimel provided motivation of using porphyrin or cyanine to measure the bio distribution in neo vasculature in CAM assay (supra). Therefore, given that many methods to measure fluorescence including FACS and confocal microscopy (as per the teaching of Brooks and Kimel) during morphogenesis were available to compare the angiogenic activity it would have obvious for an artisan to adminster porphyrin or cyanine directly into the vessel as taught by Robert/Kimel and determine fluorescence using the method of Brooks/Kimel to measure angiogenic or anti angiogenic activity as disclosed in the instant application. It is noted that the skilled Artisan would have further motivated to optimize the treatment routes, regimen and would have optimized the steps of administering test molecule in different vessel depending upon total volume of test agent required for the angiogenic or anti angiogenic response as per the teachings of Brooks (see MPEP 2144.04).

One who would practiced the invention would have had reasonable expectation of success because Brooks had already taught a method to measure angiogenic or anti angiogenic agent activity in a CAM assay. Robert et al and Kimel et al had described use of porphyrin or cyanine to measure the bio distribution in neo vasculature in CAM, which could have been used as for quantitation to compare angiogenic or anti angiogenic activity. Thus, it would have only required routine experimentation to modify the method disclosed by Brooks and Robert to include steps of administering porphyrin or cyanine directly to vessel and measure angiogenic or anti angiogenic activity as required by instant invention.

It is noted that claims 37-40 are included in the rejection because these are method steps are inherently present in order to determine fluorescence using confocal microscope as required by claim 26 (see 112 paragraph 2).

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claims 26-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Brooks et al; (Science, 1994, 264, 570-571 IDS) and Rizzo et al (Microvascular Res, 1995, 49, 49-63, IDS).

The teachings of Brooks has been discussed above and relied in same manner here. However, Brooks et al do not explicitly teach administering FITC labeled particle to measure angiogenic activity.

Rizzo et al taught a method to quantitate the relative micro vascular permeability associated with tumorigenesis and normal angiogenesis by microinjecting a graded series of FITC-dextrans into a vessel of CAM and then measuring the fluorescence by a confocal attachment to differentiate different capillary network (see page 50 bridging to page 51; materials and method). The results of Rizzo et al provide evidence that CAM could be sued as an attractive model to investigate the vasculature (see page 62, last two lines). Rizzo et al differed from the claimed invention by not teaching administering an angiogenic agent and measuring the fluorescence by confocal to measure the angiogenic or anti angiogenic activity. However, the use of confocal microscope for

measuring the angiogenic activity was known in the art at the time the claimed invention was made and these were routinely used as evidenced by Brooks et al (supra).

Accordingly, in view of the teachings of Brooks and Rizzo, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the method to measure angiogenic or anti angiogenic activity in a CAM assay of Brooks by measuring fluorescent vascular density taught by Rizzo with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as Robert had already disclosed that FITC-dextran could be microinjected to determine the vasculature (supra) and particularly since both Brooks and Rizzo et al sought to quantitate fluorescence in vasculature. Although Brooks et al did not adminster the fluorescent moiety before removing the substrate; he generally embraced potential of measuring fluorescence for quantification to better measure and compare angiogenesis. In addition, Rizzo provided motivation of using FITC-dextran to measure the neo vasculature in CAM assay because permeability of 10-day-old embryo may relect functional adaptation of the CAM (abstract). Therefore, given that many methods to measure fluorescence including confocal microscopy were available as per the teachings of Brooks amd Rizzo, it would have obvious for an artisan to adminster FITC-dextran directly into the vessel as taught by Rizzo and determine fluorescence using the method of Brooks/Rizzo to measure angiogenic or anti angiogenic activity as disclosed in the instant application. It is noted that the skilled Artisan would have further motivated to optimize the treatment routes, regimen and would have optimize the steps of administering test molecule in different

vessel depending upon total volume of test agent required for the angiogenic or anti angiogenic response as per the teachings of Brooks (see MPEP 2144.04).

One who would practiced the invention would have had reasonable expectation of success because Brooks had already taught a method to measure angiogenic or anti angiogenic agent activity in a CAM assay. Rizzo et al had already described use of FITC-dextran to measure the bio distribution in neo vasculature in CAM that could have been used as for quantitation to compare angiogenic or anti angiogenic activity. Thus, it would have only required routine experimentation to modify the method disclosed by Brooks and Rizzo to include steps of administering FITC-dextran directly to vessel and measure angiogenic or anti angiogenic activity as required by instant invention.

It is noted that claims 17-20 are included in the rejection because these are method steps are inherently present in order to determine fluorescence using confocal microscope as required by claim 1 (see 112 paragraph 2).

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claims 26-50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Brooks et al; (Methods in Molecular Biology, 129, 257-269, IDS); Kurz et al (Developmental Dynamics, 1995, 203, 174-186) and Frasca et al (Oncogene, 2001: 20, 3845-3856) as evidenced by Kinnman et al (Lab Invest. 2001; 81(12): 1709-16).

Brooks et al teach a method to measure angiogenic and anti angiogenic activity of a test molecule by obtaining a 10 day old chick egg wherein it is candled to determine

prominent blood vessel and then via a small window of exposed area a filter disc is placed followed by systemic administration of test molecule (see Figure 1pages 261-264). It is noted that Brooks et al also suggest filter disc saturated with teat agent which could be angiogenic stimulator could be placed on the CAM (see Figure 1 page 263). Brooks et al also indicate that only up to 100μl of single injection could be administered to the vessel (see page 264, paragraph 1). Brooks et al also teach quantitation of angiogenic or anti angiogenic activity by removing the filter disc and associated CAM tissue that is placed on petri dish for quantitation of number of blood vessels (see page 265, paragraph 1). While Brooks et al described the potential of measuring angiogenic and anti angiogenic activity using CAM assay. Brooks et al differed from the claimed invention by not teaching use of adding an agent to measure metabolic activity to quantitate number of viable cells in the test area.

However, prior to instant invention was made, use of proliferation-based assays was routine in the art to quantitate angiogenic or anti angiogenic activity. Kurz et al taught a method to analyze the density and distribution of whole mount BrdU anti BdU labeled endothelial cell in a CAM with computer assisted microscopy. It is noted that Kurz et al taught a method to obtain CAM at different days (see page 175, col. 2, para. 3) that were analyzed for the influence of VEGF in proliferation intensity. Kurz et al also described the nuclear incorporation (metabolic degradation) of BrdU is not as rapid in avian cells (see page 182, col. 1, para. 1, and Figure 5). It is emphasized that Kurz et al proposed that CAM endothelial proliferation is regulated by a factor such as endothelial

cell density of pre capillary vessel and length density of pre-capillary vessel, which should be used for evaluation of angiogenesis in the CAM assay (see abstract).

Kurz differed from the claimed invention by not teaching administering an agent XTT and measuring the metabolic activity to measure the metabolic activity of cell in the test area. However, the uses for XTT, MTT, WST-1or BrdU for measuring the cell proliferation was known in the art at the time the claimed invention was made and these assay were routinely used in alternative to each other.

Frasca et al taught a method of determining the effect of a synthetic molecule STI571 on HGF-induced morphogenesis. It is noted that Frasca taught a method to determine the proliferation of cells on matrigel to determine the effect of test agent on proliferation during morphogenesis. Frasca et al describe addition of an agent XTT after the addition of test molecule and described the method to measure and compare the metabolic activity at a specific wavelength (450nm) as described by the manufacturer (Roche Laboratory see page 3855, col. 1, para. 3, and Fig. 3). In addition, Kinnman provided evidence that cell proliferation/ cell viability could be measured by multiple method including BrDu, XTT and cell counting (see page 1711, Figure 5). However, Frasca et al do not explicitly teach a method of comparing proliferation of cells during the formation of capillary in a CAM assay.

Accordingly, in view of the teachings of Brooks and Kurz, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the method to measure angiogenic or anti angiogenic activity in a CAM assay of Brooks by measuring endothelial cell density with a reasonable expectation of

success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as Kurz had already disclosed that proliferative pattern and the length density and extension should be used for the evaluation of angiogenesis in the CAM (see page 174, abstract, last 5 lines) and particularly since both Kunz and Brooks et al sought to quantitate angiogenic or anti angiogenic activity. Although Brooks or Kurz et al did not use XTT, Kurz generally embraced potential of measuring proliferation assay to better measure and compare angiogenesis. In addition, Kurz provided motivation of measuring proliferation of cells to measure the vessel density and length for quantitation of angiogenesis in CAM assay (supra). Therefore, given that many methods to measure proliferation of cell including XTT, Brdu were available for determining the proliferation of cells during morphogenesis to compare the angiogenic activity of test molecule as per the teachings of Frasca and Kinnman it would have obvious for an artisan to use XTT or any other assay to determine cell viability/proliferation assay or metabolic activity to measure angiogenic or anti angiogenic activity as disclosed in the instant application. It is noted that the skilled Artisan would have further motivated to optimize the treatment routes, regimen and would have optimize the steps of administering test molecule and agent to measure metabolic activity in different vessel depending upon total volume of test agent required for the angiogenic or anti angiogenic response as per the teachings of Brooks (see MPEP 2144.04).

One who would practiced the invention would have had reasonable expectation of success because Brooks had already a method to measure angiogenic or anti

angiogenic agent activity in a Cam assay. Kurz and Frasca had already described use of proliferation/viability assay to determine proliferation pattern during morphogenesis that could have been used as for quantitation to compare angiogenic or anti angiogenic activity. Thus, it would have only required routine experimentation to modify the method disclosed by Brooks and Kurz to include XTT assay to measure angiogenic or anti angiogenic activity as required by instant invention.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Double Patenting

A rejection based on double patenting of the "same invention" type finds its support in the language of 35 U.S.C. 101 which states that "whoever invents or discovers any new and useful process ... may obtain a patent therefor ..." (Emphasis added). Thus, the term "same invention," in this context, means an invention drawn to identical subject matter. See *Miller v. Eagle Mfg. Co.*, 151 U.S. 186 (1894); *In re Ockert*, 245 F.2d 467, 114 USPQ 330 (CCPA 1957); and *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970).

A statutory type (35 U.S.C. 101) double patenting rejection can be overcome by canceling or amending the conflicting claims so they are no longer coextensive in scope. The filing of a terminal disclaimer cannot overcome a double patenting rejection based upon 35 U.S.C. 101.

Claims 26-50 provisionally rejected under 35 U.S.C. 101 as claiming the same invention as that of claims 1-2, 7-22 and 27-35 of copending Application No. 11/014472. It is noted that both sets of claims are directed to a method of measuring the angiogenic or anti angiogenic activity of a test molecule in a CAM assay by administering a

fluorescent-labeled particle and measuring the FVD value or by using an agent that has metabolic activity and measure spectrophotometer reading to determine angiogenic activity. Since the specification and claims of the '472, application contemplated same test molecule and fluorescent-labeled particle or by using XTT and embraced same method steps in CAM assay as one disclosed in instant application.

This is a provisional double patenting rejection since the conflicting claims have not in fact been patented.

Conclusion

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Brooks et al (Methods in Molecular Biology, 129, 257-269, 1999, IDS).

No Claims allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anoop Singh whose telephone number is (571) 272-3306. The examiner can normally be reached on 9:00AM-5:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272- 0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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